# MAQC Main Study – Illumina Experimental Plan

## Test Sites

### *(1) Illumina*

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## Required Materials

### *Equipment*

Safety glasses
Lab coats
Single-channel precision pipet, p2, p20 and p100
Tube vortexer
Table-top centrifuge with 96-well plate carriers (for drying slides)
Microcentrifuge (strip tube compatible)
Vacuum centrifuge concentrator (strip tube compatible)
NanoDrop ND-1000A UV-Vis Spectrophotometer (or equivalent device for measuring RNA OD260/280)
Agilent 2100 Bioanalyzer
Thermal cycler with adjustable-temperature heated lid–or–hybridization ovens set at 42ºC, 37ºC and 16ºC heat block
Orbital shaker
Rocker mixer

Scigene Hybex™ Microarray incubation system with waterbath insert
Compressed air source (at least 50 psi) for isolation table
Software: BeadScan 2.3.0.10 and BeadStudio 1.5.1.3

### *Disposables*

Clean paper towels
RNAse-free 0.5 ml microfuge tubes (Note: we recommend the use of strip tubes when you are labeling multiple samples.)
RNAse-free 15 ml disposable conical tubes (for blocking and SA-Cy3 steps)
Powder-free protective gloves

### *Kits and Reagents*

Illumina RNA Amplification Kit (Ambion, Inc., catalog # I1755) – **supplied by Illumina**
Biotin-16-UTP, 10mM (Perkin Elmer) – **supplied by Illumina**
Illumina High-temp wash buffer (Illumina part number 11206039) – **supplied by Illumina**
Four (4) Sentrix Human-6 Expression BeadChips, 48k v1.0 (Illumina, catalog# BD-25-101) – **supplied by Illumina**
Human-6 buffer kit (Illumina, included with chips) – **supplied by Illumina**
Control RNA sample – **supplied by Illumina**
Ambion's Human Brain Reference RNA (HBRR) – to be delivered by Ambion
Stratagene's Universal Human Reference RNA (SUHRR, Catalog number: 740000) – **to be delivered by Ambion**
Mixture #1 (75% HBRR/25% SUHRR) – **to be delivered by Ambion**
Mixture #2 (25% HBRR/75% SUHRR) – **to be delivered by Ambion**
100% ethanol
Deionized formamide (Fischer Scientific, catalog # 4610)
Streptavidin-Cy3 ( FluoroLink™ Cy™3, Amersham Biosciences catalog # PA43001, diluted to 1mg/mL or 1 µg/µL with RNase-free water ); NOTE: Once the SA-Cy3 has been reconstituted, Illumina recommends making 20 µL aliquots. Store these aliquots at -20°C. Once the aliquots have been thawed for use, store them in the dark at 4°C for up to one month. DO NOT FREEZE AND THAW REPEATEDLY.
RNase-free water

## Experimental Design

Five (5) replicate sample labelings and hybridizations will be performed on the following four (4) tissue mixtures:
SUHRR (A)
HBRR (B)
25% HBRR/75% SUHRR (C)
75% HBRR/25% SUHRR (D)

## Sample Preparation

All samples should be processed at the same time by the same person.

### *Total RNA QC*

The SUHRR sample will be delivered in 70% ethanol and 0.1M sodium acetate and should be prepared according to Stratagene's recommended procedure. The HBRR and mixture samples will be delivered as aqueous solutions. For Site 2, all samples will be delivered by Illumina as aqueous solutions. Before

proceeding to the sample labeling step, all total RNA samples should be quantitated with a NanoDrop spectrophotometer (or equivalent) and the OD260 and OD280 measurements should be recorded and reported to the MAQC study. (See “MAQC Sample Processing Overview” document for details.)
A quality assessment should be made by running 200ng of each of the four samples on the Bioanalyzer system. The Bioanalyzer traces should be saved along with the 28S/18S ratios and RIN values. (See “MAQC Sample Processing Overview” document for details.)

### *Sample Labeling*

All 20 sample labeling reactions should be performed in parallel by the same operator. For each replicate, 200ng total RNA should be labeled according to the procedure described in the manual included in the “Illumina® RNA Amplification Kit”. The IVT reaction should be run for 16 hours.

### *Labeled cRNA QC*

Before proceeding to the sample hybridization step, all cRNA samples should be quantitated with a NanoDrop spectrophotometer (or equivalent) and the OD260 and OD280 measurements should be recorded and reported to the MAQC study. (See “MAQC Sample Processing Overview” document for details)
The fragment size distributions should be made by running 200ng of each of the 20 replicates on the Bioanalyzer system using the Eukaryotic mRNA Assay with smear analysis. The Bioanalyzer traces should be saved. (See “MAQC Sample Processing Overview” document for details.)

## Sample Hybridization

Samples should be prepared for hybridization according to the “Gene Expression on Sentrix® Arrays Direct Hybridization System Manual” with the addition of a ten-minute wash in Illumina High-temp wash buffer at 55°C for 10 minutes in a Scigene Hybex Microarray Incubation System with a waterbath insert following the overnight hybridization. All hybridizations should be performed in parallel by the same operator.
1.5ug cRNA should be used for each hybridization.
The following sample layout should be used:

| Chip 1 | Chip 2 | Chip 3 | Chip 4 |
|---|---|---|---|
| Control | B2 | C3 | D4 |
| A1 | Control | D3 | A5 |
| B1 | C2 | Control | B5 |
| C1 | D2 | A4 | Control |
| D1 | A3 | B4 | C5 |
| A2 | B3 | C4 | D5 |

“Control” is an Illumina control sample to be used only for internal purposes (it is not part of the MAQC study and those samples should not be reported).
Hybridizations should be carried out for 16 hours at 55oC.
All four slides should be washed and processed in parallel.

## Scanning

The chips should be scanned as soon as possible after the final dry down procedure using BeadScan 2.3.0.10 at a multiplier setting of two (2).
The slides should be scanned in order with chips 1, 2 and 3 batched together in the first scanner run and chip 4 in the second scanner run.

# Data Analysis/Outputs

The microarray images will be registered and extracted automatically during the scan according to the manufacturer's default settings.
BeadStudio version 1.5.1.3 should be used. The samples should be named according to the standard MAQC convention (e.g., A1, A2, A3, A4, A5, B1, etc.) and generated twice in two rounds of processing. The first processing should use no normalization. The second should use the cubic spline normalization method. The reference group for the normalized experiment should be a group containing all of the brain samples (B1, B2, B3, and B4). Because this group is only used for the sake of normalization, all columns pertaining to the group should be deleted from the gene_profile file prior to data submission. For each experiment, each site should generate standard text output containing the following fields:

- AVG_Signal
- BEAD_STDEV
- Avg_NBEADS
- Detection

The naming conventions for the files shall be as follows:

| Site | Normalized file name | Raw data file name |
|---|---|---|
| Illumina | ILM_1_ALL_norm_gene_profile.csv | ILM_1_ALL_raw_gene_profile.csv |
| UT Southwestern | ILM_2_ALL_norm_gene_profile.csv | ILM_2_ALL_raw_gene_profile.csv |
| Burnham Institute | ILM_3_ALL_norm_gene_profile.csv | ILM_3_ALL_raw_gene_profile.csv |

For each site, these files plus the additional files generated by the scanner (.tif, .IDAT, .locs, .sdf, .csv and .xml) will be zipped into a Winzip compressed file and submitted to the MAQC FTP site per instructions provided by Leming Shi.

In addition, the normalized file shall be processed outside of the BeadStudio software to eliminate negative hybridization signal values by adding an offset constant. This constant will be chosen such that the lowest AVG_Signal value for each sample has a value of 1. The processing is necessary because some of the analyses to be conducted by the MAQC group will not work with negative values. Because this processing occurs outside of the normal BeadStudio software, it will be conducted by Illumina using data provided by the third-party sites.